Research Thesis: Aptamer Inhibition of von Willebrand Factor in Canine Myocardial Infarction

Presented in partial fulfillment of the requirements for graduation *with Research Distinction* in *Neuroscience* in the undergraduate colleges of The Ohio State University

Caitlin M. Hatten

The Ohio State University May 2020

Project Advisor: Dr. Shahid Nimjee, MD, PhD Associate Professor, Department of Neurological Surgery Wexner Medical Center at The Ohio State University

TABLE OF CONTENTS

I. Abstract

Heart disease is the leading cause of death for both men and women worldwide[1]. With this project, we propose a new therapy to provide a safer and more effective thrombolytic for patients who suffer an MI. von Willebrand Factor (VWF) is a known mediator in platelet adhesion and aggregation. We have developed an RNA aptamer which acts to directly inhibit von Willebrand Factor. This aptamer, named DTRI-031, can be reversed with an antidote oligonucleotide, DTRI-025, that was specifically developed to rapidly and durably reverse DTRI-031 function. Previous studies in this laboratory have demonstrated that DTRI-031 resulted in decreased infarct sizes after stroke and decreased platelet reactivity after vascular injury in canines. Our current study will first attempt to modify a large animal model of porcine myocardial infarction (MI) for canine utilization. Once our canine MI model is characterized, we will attempt to demonstrate the ability of DTRI-031 to maintain cardiac function and platelet reactivity after MI compared to vehicle control. We will utilize cardiac magnetic resonance imaging (MRI) to measure ejection volume, stroke volume, and cardiac output. We will stain hearts with Evan's Blue after MI to compare infarct size. Lastly, we will monitor platelet reactivity utilizing whole blood aggregometry before MI, during ischemia, and at time of sacrifice in addition to measurement of plasma VWF levels. Heart rate, body temperature, and blood pressure monitoring ensure physiological conditions are maintained throughout the surgical procedures. We anticipate that the canine MI model will be characterized rapidly with little modification from the previously published porcine model and that DTRI-031 will result in less infarction and retain greater cardiac function. This is an ongoing experiment.

II. INTRODUCTION

A. Historical Background

According to the Centers for Disease Control and Prevention, the leading cause of death of men and women is heart disease. In the United States, almost every 1 in 3 deaths each year is from cardiovascular disease, with a heart attack occurring every 40 seconds[1]. Heart attack, or acute myocardial infarction (AMI) can occur when a blood vessel in the heart is blocked and muscle is damaged due to lack of oxygen[2]. According to the National Institutes of Health's (NIH) National Heart, Lung and Blood Institute, there are treatments available for suspected heart attacks which include: aspirin and nitroglycerin. These treatments are used in order to prevent thrombosis or relax blood vessels, respectively to maintain blood flow. If MI does occur, thrombolytics, anticoagulants and other non-surgical interventions are used. Reperfusion therapies decrease the morbidity rate of AMI[3]. Unfortunately, Sánchez-Hernádez et al describe studies that have shown how blood flow restoration results in an inflammatory response which can further the damage[4]. In summary, there is an immediate need for treatments which utilize novel targets to reduce infarct size and preserve cardiac function.

B. Hemostasis

Understanding blood hemostasis and how a blood clot forms is important to understand MI and MI treatment strategies. Complications from platelet hyperactivity in the first stages of an AMI are common and related to platelet aggregation, ultimately resulting in thrombosis[5]. Subendothelial components are exposed at sites of injury in vasculature. These components are a key factor in aggregation[6]. Tomaiuolo et al illustrates a model of the hemostatic response to an injury in the vasculature [7]. The platelet plug formation requires a cascade of events of platelet

aggregation and activation. Interactions and adhesion factors initiate the aggregation, followed by platelet activation at that site and stabilization of the activated platelets in a core region. The adhesion of platelets to the injury sites can lead to excessive accumulation and result in thrombus formation which may lead to AMI. Many factors are involved in this process. Studies have shown that von Willebrand Factor (VWF) plays a role in platelet aggregation and thrombus formation[6,7,8] **(Figure 1).**

C. von Willebrand Factor

VWF has recently become an important factor in clot formation. VWF is a glycoprotein and each subunit has multiple binding sites, including binding sites for platelet glycoprotein Ib (GPIb) and GPIIb/IIIa[9] (Figure 2). GPIb is a receptor found on the surface of platelets and it functions as a meditator for the first step in platelet adhesion. It facilitates the binding of VWF under injury conditions[31,32]. GPIIb/IIIa is an integrin found on platelets. It has a receptor for VWF and aids in the activation of platelets[33,34]. VWF is produced by endothelial cells and megakaryocytes and are stored in Weibel Palade bodies within the endothelium and platelet a-granules, respectively. They are released upon injury to the vessel in cases like hypoxia or during endovascular procedures such as angioplasty and thrombectomy[8,10]. During injury, VWF interacts with platelets through the GPIb-IX-V receptor and GPIIb-IIIa receptor[6]. GPIba binds to VWF at the A1 domain which is exposed during high arterial shear rates such as atherosclerosis and MI. This binding is not found in static conditions. This binding by the GPIb-IX-V complex plays a major role in aggregation[6,8,11]. von Willebrand Disease (VWD) is characterized by a reduction in VWF in blood plasma. The predominant form of VWD, called Type 1 VWD, presents itself with uncommon and increased bleeding during dental procedures and menorrhagia in women[12]. Patients

with VWD have been shown to be more protected from cardiovascular and cerebrovascular events[13]. Following AMI, acute ischemic stroke (AIS) and cardioembolic stroke, patients have increased levels of plasma VWF[13-18].

VWF also plays a significant role in inflammation as it relates to thrombosis. A study done by Andreotti et al demonstrates the rapid changes in plasma following tissue damage and states that VWF levels were higher in the first 48 hours after infarction compared to the concentrations after 90 days[19]. There is a high rate of platelet adhesion to the subendothelium following ischemia reperfusion (I/R) at the injury site due to the excess exposed VWF which can increase myocardial infarct size. The inflammatory response is enhanced and can lead to a higher tendency for vessels to re-occlude in the same area.[20] Until recently, VWF has been overlooked as a suitable target for therapeutics[13] and the inhibition of VWF could show potential. With that, Dr. Nimjee's group has isolated an RNA aptamer, DTRI-031, to inhibit VWF[10] by binding to the A1 domain of VWF **(Figure 2)**.

D. DTRI-031

Aptamers are DNA or RNA ligands that act as inhibitors that bind to and inhibit a target protein with high affinity and specificity. Aptamers are created by SELEX, (Systematic Evolution of Ligands by EXponential enrichment), an in-vitro selection technique developed by Tuerk and Gold, and Ellington and Szostak[13,21,22] **(Figure 3)**. There are many advantages of using aptamers including the ability to modify their half-life, unlimited shelf life and low to no immunogenicity. Also, they can be isolated to any protein and have high specificity towards that target[23]. A matched antidote, DTRI-025, was also created using the properties of Watson-Crick base-pairing **(Figure 4)**. DTRI-025 binds and inhibits DTRI-031 which is imperative as with any drug that prevents or

treats thrombosis, such as treatments for AMI and AIS that have a risk of hemorrhage caused by current thrombolytic drugs[10].

E. Project Overview

We previously tested the efficacy of DTRI-031 in preventing an occlusive thrombus[12]. More recently, we have demonstrated that DTRI-031 can recanalize a formed clot better than rTPA, the only approved thrombolytic drug. The same model was also utilized to study the efficacy of DTRI-031 to thrombolyse stabilized blood clots in both mice and canines[12,30] . In both of these small and large animal models of vascular injury, VWF inhibition by DTRI-031 resulted in greater thrombolysis.

My project will determine whether DTRI-031 efficacy translates into myocardial infarction, another disorder caused by thrombosis. I will first attempt to translate a previously performed MI model from porcine to canine then evaluate the effect of DTRI-031 on the preservation of cardiac function after MI. Animals will be subjected to 60 minutes of ischemia induced by balloon insertion and inflation in the LAD followed by reperfusion and infusion with either 0.5mg/kg DTRI-031 (5 minute bolus) or vehicle for an additional 60 minutes. Whole blood will be drawn for platelet aggregometry (Chronolog) and VWF quantitation at designated times and as well as digital subtraction angiography performed to monitor cardiac perfusion. To evaluate infarction size and cardiac function, cardiac MRI will be performed 120 minutes after treatment infusion and Evans Blue staining will be performed at the time of sacrifice to verify infarct size. We anticipate that DTRI-031 will result in a smaller infarct size and will retain greater cardiac function.

III. Materials and Methods

Animal procedures were approved by The Ohio State University Institutional Animal Care and Use Committee (IACUC). Five adult canines (<1year old) were obtained from Covance and housed in a temperature and humidity-controlled vivarium in Wiseman Hall with free access to food and water **(Table 1)**.

In vivo myocardial injury: Canine AMI were performed as previously published[21]. A model of myocardial ischemic reperfusion (IR) infarction using a simple catheterization procedure to occlude the mid left anterior descending artery (LAD) was used. Adult beagles, both male and female, 8-12kg were fasted overnight with free access to water before MI. A pre-anesthetic intramuscular administration of acepromazine (0.2 mg/kg) was delivered and a 20 gauge catheter was introduced into a cephalic vein. Anesthesia was induced with intravenous administration of ketamine (10 mg/kg) and midazolam (0.025 mg/kg). Following induction, dogs were intubated and mechanically ventilated using constant inhaled isoflurane for anesthesia maintenance. The dogs were positioned supine and an arteriotomy was performed to expose either the right femoral or left carotid artery. A 7F arterial sheath was introduced into the isolated vessel for access and invasive blood pressure measurements. To assess baseline hemodynamics, a 6F pig-tail catheter was placed into the sheath and advanced via a .035" guide wire into the left ventricle. As an antiarrhythmic, an intravenous bolus of amiodarone (30 mg) and lidocaine (20 mg) was administered prior to angiograms. The pig-tail catheter was removed and replaced with a 6F IM or RCB guiding catheter (Cordis, Miami Lakes, FL) which was advanced to the left main coronary ostium. Digital angiography was obtained of the target vessel prior to the initiation of ischemia. A 2.5 mm x 15 mm coronary angioplasty balloon catheter (Boston Scientific, Natick, MA) was positioned in the mid-LAD. The balloon was inflated to nominal pressure preventing

blood flow to the distal coronary to induce ischemia. Total occlusion of the coronary artery distal to the balloon was verified by angiogram. EKG and blood pressure were closely monitored during the ischemic period. Episodes of ventricular fibrillation were cardioverted using nonsynchronized direct current defibrillation set at 200J. After 60 minutes of ischemia, the balloon was deflated and removed from the vessel. A follow up angiogram was performed to verify restoration of blood flow in the previously occluded artery. Thrombolytic therapy was then initiated.

Angiography: Canine digital subtraction angiography (DSA) was performed as previously published[21]. Coronary angiography of the target vessel was conducted with a 7-Fr guide catheter (AL1 STSH Guide, Boston Scientific, Natick, MA) advanced into the left main coronary ostium. A coronary angioplasty balloon, catheter sized (Sprinter OTW 4.0x10 mm balloon, Medtronic Inc., Minneapolis, MN) according to the target vessel, was positioned in the left anterior descending artery (LAD) proximal to the first diagonal artery branch. To induce ischemia, the balloon was inflated to nominal pressure and cessation of blood flow to the distal LAD was confirmed with DSA. Episodes of ventricular fibrillation were cardioverted using nonsynchronized direct current defibrillation set at 360J. After 60 minutes of ischemia the balloon was deflated, removed from the vessel, and restoration of flow verified with an angiogram. The animals were monitored for an additional 3 hours and a concluding hemodynamic assessment, left ventriculogram, and coronary angiogram were recorded[21].

Infarct Size Assessment: Determination of cardiac infarct size was performed as previously published[21]. Briefly, after MRI and immediately before sacrifice, the angioplasty balloon was re-inflated into the same position in the LAD. The heart was exposed by a median sternotomy and

the LAD was ligated at the proximal end of the inflated angioplasty balloon. The heart was excised and perfused with 40-60 ml of 10% Evan's Blue injected directly into the left and right coronary artery ostia for delineation of the area-at-risk (AAR) for myocardial infarction from the non-ischemic zone. The area of the myocardium that did not stain with Evan's Blue was defined as the AAR[21].

Chronolog and VWF Activity: Canine whole blood was collected per experimental time points at baseline before MI, immediately after ischemia (Baseline 2), 5 minutes, 15 minutes, 30 minutes, 60 minutes, 90 and 120 minutes after treatment infusion. Whole blood was also collected at time of sacrifice. Whole blood was collected for anti-coagulation in sodium citrate tubes for VWF levels and lithium heparin tubes for whole blood aggregometry (WBA). Blood for WBA was diluted with sterile saline in a 1:1 ratio and rested for 30 minutes in an incubator at 37 degrees Celsius. Platelet impedance with collagen, ADP, and botrocetin was measured for 6 minutes using a Chronolog platelet aggregometer.

Imaging: Magnetic Resonance Imaging (MRI) was performed as previously published in the Large Animal Catherization Core at baseline and immediately before sacrifice[,23]. The infarct size in the dogs was imaged as previously published[13,21,22].

IV. Results

As previously discussed, our first goal was to characterize canine MI based on the porcine model. The vehicle treatment group thus far has consisted of 5 adult dogs. We successfully completed baseline MRI on four of the five dogs prior to the day of the MI procedure. Of the five animals on which we initiated the MI protocol, one canine survived throughout MI and MRI to experimental time of sacrifice. Experimental protocol consisted of 60 minutes of ischemia,

followed by vehicle infusion, 120 minutes of monitoring, MRI and sacrifice for tissue harvest[21]. Dog #M1, CIJCKG died after baseline. CIJCKG experienced complications with placing the balloon in the LAD. Dog #M2, CIICPE received 60 minutes ischemia, vehicle infusion, and completed 120 minutes of monitoring but died 46 minutes into MRI scanning. Dog #F1, CIICTJ died after baseline data collection at the beginning of the MI protocol probably due to anatomical abnormalities identified during the baseline MRI. Dog #F2, CIICTL, completed the MI protocol **(Figure 5)**.

Functional Differences:

To demonstrate the translation of the porcine MI procedure to canines, functional differences of ejection volume, cardiac output and stroke volume were evaluated pre-MI and post-MI ($p<0.05$). Ejection fraction (EF) is the measurement of how much blood (% of total blood) the left ventricle pumps out in one contraction[25]. The ejection fraction of pre-MI (52.375%+/-13.093%, n=4) and of post-MI (32.880%, n=1) were measured **(Figure 6)**. Cardiac output (CO) is the total amount of blood that the heart pumps out in one minute[27]. The cardiac output of pre-MI (18.613+/-11.616 L/min, n=4) and of post-MI (19.830 L/min, n=1) were measured **(Figure 7)**. Stroke volume (SV) is the amount of blood that is ejected from the left ventricle in a heart beat[27]. The stroke volume of pre-MI (15.755+/-4.139 mL, n=4) and of post-MI (6.080 mL, n=1) were measured **(Figure 8)**.

Infarct Size:

To demonstrate the efficacy of DTRI-031 the infarct size was visually assessed[21]. As stated previously, after organ harvest, the heart was removed and perfused with 40-60mL of 10% Evan's Blue (Sigma Aldrich, St Louis, MO). The area that did not stain blue was defined as the Area-at-

Risk (AAR). We performed infarct assessment on Dog #F2 CIICTL (**Figure 9**). The infarct size of the heart has not quantitatively measured.

Physiological Effects:

Heart rate (HR) and blood pressure (BP) were monitored throughout the experiment and reported at baseline before MI, immediately after ischemia (Baseline 2), 5 minutes, 15 minutes, 30 minutes, 60 minutes, 90 and 120 minutes after treatment infusion and then immediately before sacrifice. Normal physiological conditions were maintained throughout those time points. Statistical analysis of the data collected was done using a one-way ANOVA for comparison across all time points. Baseline heart rate between animals was not significant (n=5, p=0.1341) **(Figure 10)**. Baseline mean blood pressure between animals was not significant (n=5, p=0.7253) (**Figure 11**). Baseline systolic blood pressure (n=5, p=0.3334) and diastolic blood pressure (n=5, p=0.8498) between animals was not significant **(Figure 12)**.

Platelet Reactivity:

Platelet reactivity was recorded. Aggregation impedance with collagen, ADP, and botrocetin was measured for 6 minutes using a Chronolog whole blood aggregometer. Collagen polymerizes into fibrils for platelet activation[28]. The concentration of collagen used was 3.2μg/ μL. Statistical analysis per treatment group was done using a one-way ANOVA for comparison across all time points for each parameter: amplitude (ohms), slope (ohms per minute), area under the curve (AUC, ohms*time, in % of impedance) and lag time (minutes). The resulting amplitude in response to collagen showed no significance (n=3, p=0.6349). The resulting slope in response to collagen showed no significance (n=3, p=0.4441). The resulting AUC in response to collagen showed no significance (n=3, p=0.7534). The resulting lag time in response to collagen showed

no significance (n=3, p=0.0758) (**Figure 13**). Adenosine diphosphate, ADP, exposes the fibrinogen-binding site[28]. The ADP concentration used was 20μM. The resulting amplitude in response to ADP showed no significance (n=3, p=0.0007). The resulting slope in response to ADP showed no significance (n=3, p=0.7637). The resulting AUC in response to ADP showed no significance (n=3, p=0.0001). The resulting lag time in response to ADP showed no significance (n=3, p=0.3098) (**Figure 14**). Botrocetin contains a sequence analogous to GPIb which means it can be used to indirectly measure VWF activity. The botrocetin concentration used was 1 μg/ μL. The resulting amplitude in response to botrocetin showed no significance (n=3, p=0.0948). The resulting slope in response to botrocetin showed significance (n=3, p=0.0472). The resulting AUC in response to botrocetin showed no significance (n=3, p=0.4139). The resulting lag time in response to botrocetin showed no significance (n=3, p=0.8635) (**Figure 15**).

V. Discussion

Functional Differences:

We found there was no significance between animals at baseline in any functional parameter. Thus far in these experiments, the pre-MI data and mean +/- standard deviation were collected from 4 canines, while the post-MI data resulted from only one canine at time of sacrifice. We believe we have translated the previous published porcine model to the canine and have adequately characterized the canine model by MRI optimization to be utilized for DTRI-031 efficacy studies. In the future, we hope to increase this sample size in the negative control group. Unfortunately due to the time restrictions we were unable to begin DTRI-031 treated canines to determine functional differences and therefore unable to analyze and determine the efficacy of DTRI-031 compared to the negative control. With that, we anticipate not only a significance in pre- and post-MI differences with DTRI-031 treatment in maintaining cardiac function, but a significant preservation compared to the negative control.

Infarct Size:

The infarct size from one canine was visually assessed from the negative control group. Unfortunately we do not yet have DTRI-031 treated hearts for comparison and therefore are unable to analyze and determine the efficacy of DTRI-031 against the negative control. For the future of this study we plan to complete the negative control group as well as compare with DTRI-031 treatment. When both the negative control and DTRI-031 hearts have been sectioned and stained, the same investigator blinded to treatment will trace and analyze all hearts for consistency and accuracy. With that, we anticipate a significantly smaller infarct size with DTRI-031 treatment consistent with maintenance of cardiac function compared to the negative control.

Physiological Effects:

Analysis of heart rate and blood pressure in the animal data thus far suggests that normal physiological conditions were maintained throughout the experiment for the negative control group (n=5). Unfortunately we do not yet have physiological data from DTRI-031 treated canines for comparison and therefore are unable to analyze and determine if there is a difference compared to the negative control. For the future of this study we plan to increase the sample size of the negative control as well as compare with DTRI-031 treatment. With that, we anticipate no significant difference in physiological data with DTRI-031 treatment consistent with our previous experiments in other canine models where we have administered DTRI-031 intravenously compared to the negative control.

Platelet Activity

The platelet reactivity resulting from agonist addition including collagen, ADP and botrocetin were recorded for the negative control group only thus far. Chronolog whole blood aggregometry was used to determine the impedance of the clot formation. As stated previously, statistical analysis for the negative control was done using a one-way ANOVA for comparison across all time points for each parameter including amplitude (ohms), slope (ohms per minute), area under curve (AUC, ohms*time, in % of impedance) and lag time (minutes). One-way ANOVA was used due to the small sample size and the inability to run t-tests with specific time points. There was no noted difference across all agonists and each parameter except for the slope when using botrocetin. Botrocetin is used for the detection of VWF, which is of major importance to this study specifically due to DTRI-031 inhibition of VWF. This is likely due to the increased levels of VWF present in blood plasma after an ischemic injury[8,10]. In the future, we plan to

increase this sample size in the negative control. Unfortunately due to the time restrictions we were unable to begin DTRI-031 treated canines to determine differences in platelet reactivity and therefore unable to analyze and determine the efficacy of DTRI-031 compared to the negative control platelet activation and aggregation. With that, we anticipate a significant difference in platelet reactivity induced by botrocetin addition with DTRI-031 treatment consistent with our previous experiments in other canine models where we have administered DTRI-031 intravenously compared to vehicle treatment. Specifically, we believe we will see DTRI-031 blunt or entirely eliminate platelet activation and aggregation immediately after infusion begins.

Our canine MI research is expected to continue in hopes to gather more data to accurately assess whether DTRI-031 can translate from a stroke to an MI therapeutic. We plan to further our collection of the data above by completing the negative control group. We also plan to being using DTRI-031 treated animals and compare DTRI-031 to the negative control. In the future, we plan to complete ELISAs, Enzyme Linked Immunosorbent Assays, to measure concentrations of VWF and ADAMTS13 as it is important for VWF size regulation, and inhibition of VWF by DTRI-031 should increase this concentration[29].

VI. Figures:

Figure 1

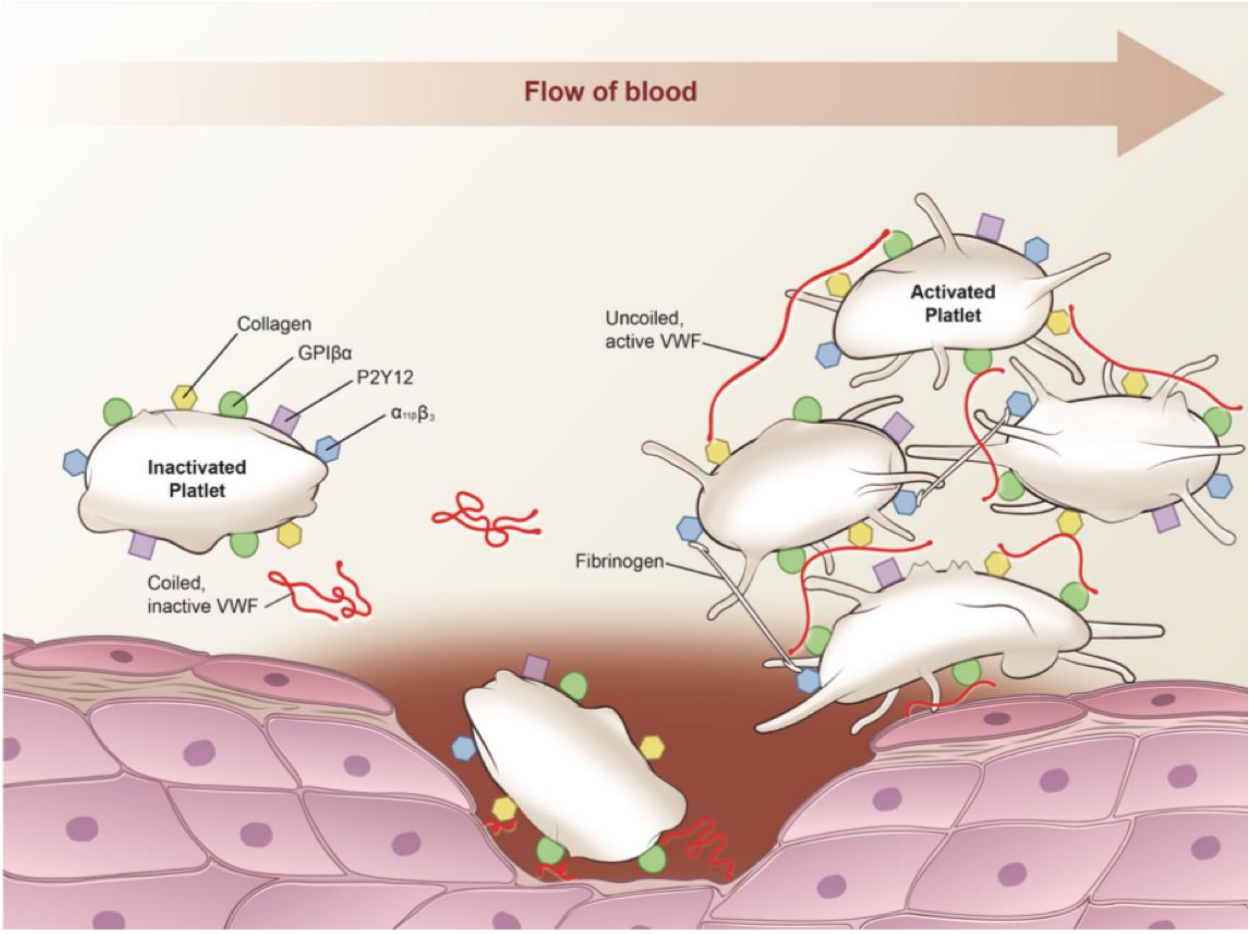


Figure 1: Role of VWF in platelet aggregation and thrombus formation (Nimjee et al. 2020)

Figure 2

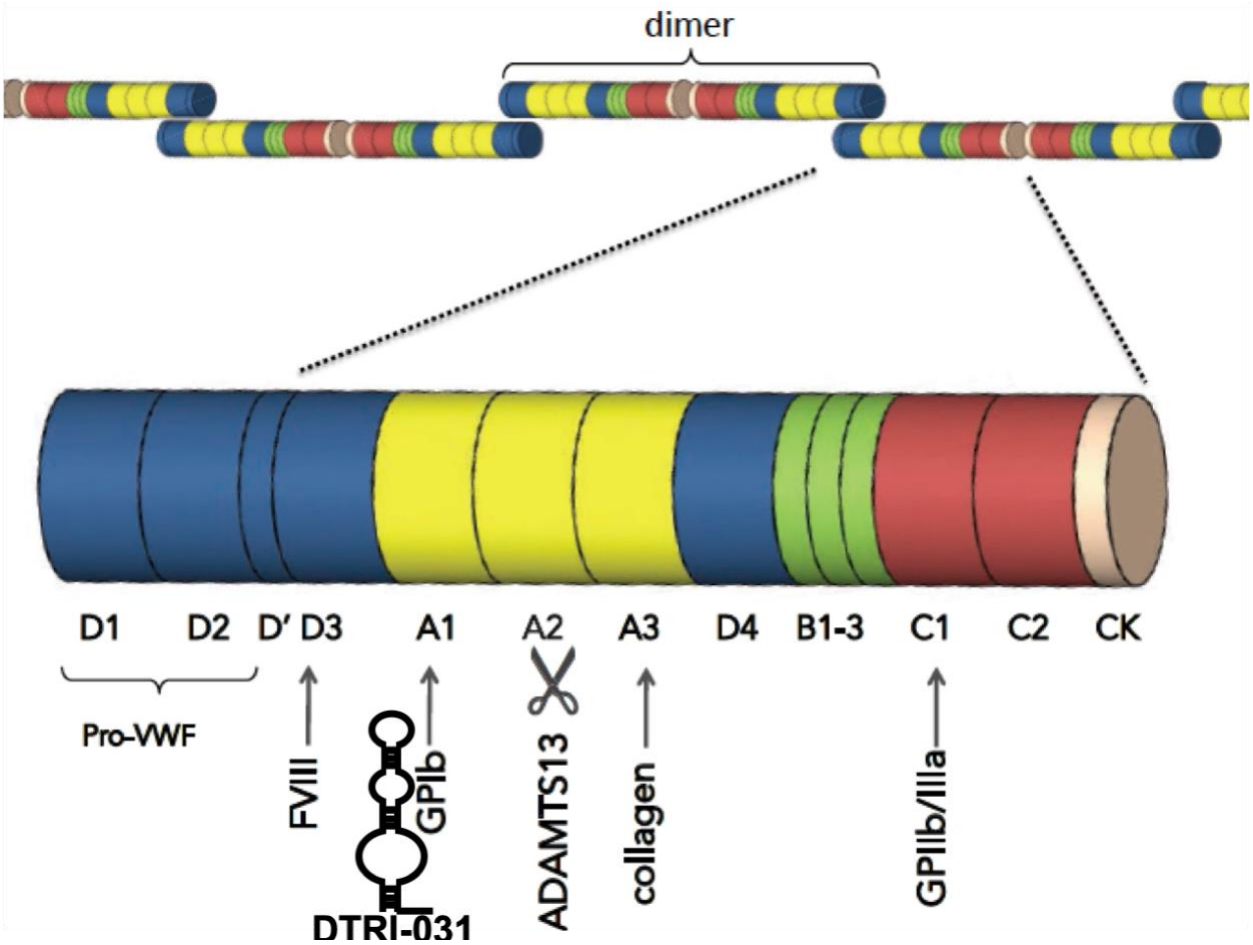


Figure 2: The structure of von Willebrand Factor, VWF. The A1 domain is where the aptamer DTRI-031 binds which is the GPIb binding site. (Nimjee et al 2020)

Figure 3

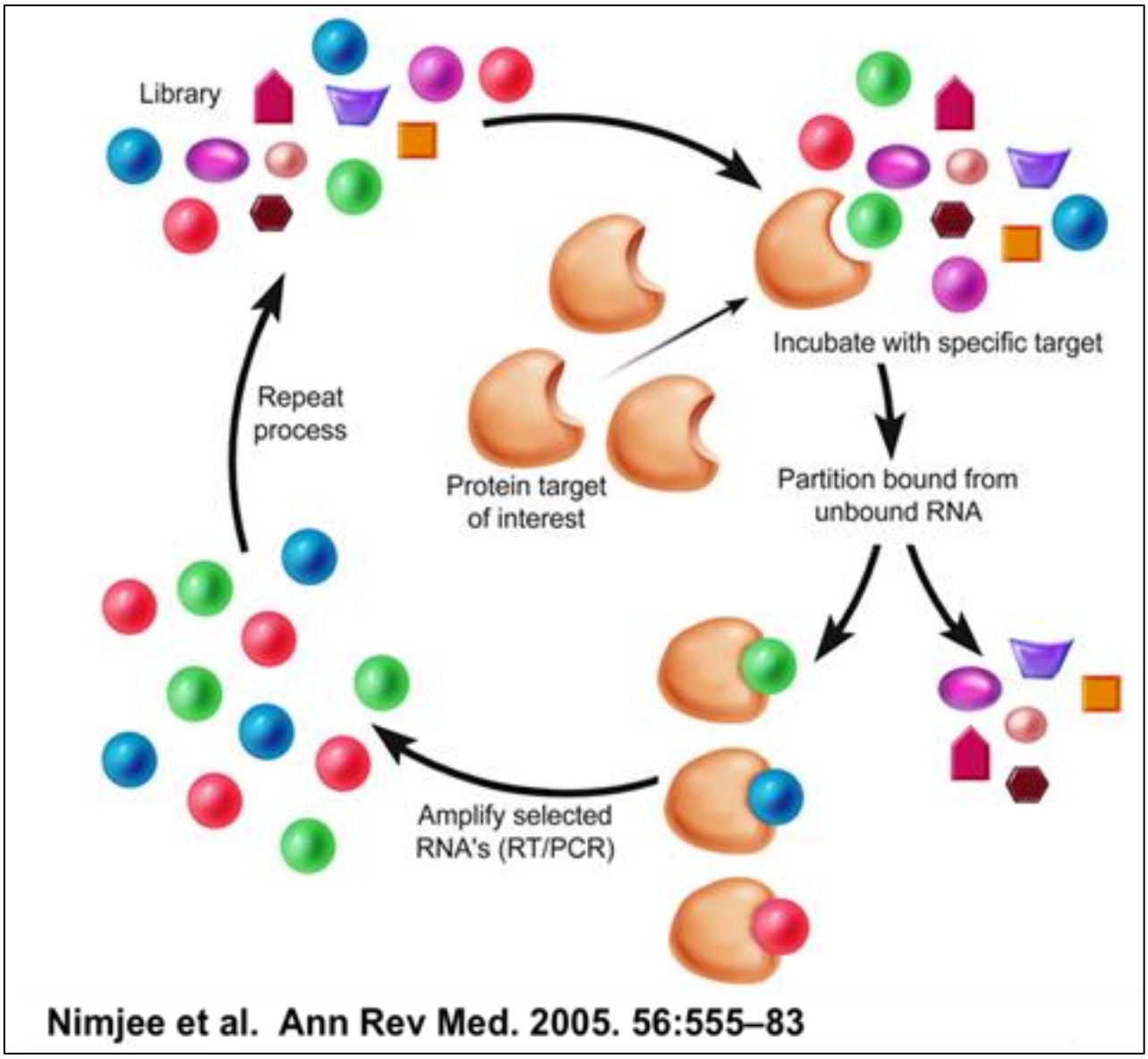


Figure 3: SELEX Method

Figure 4

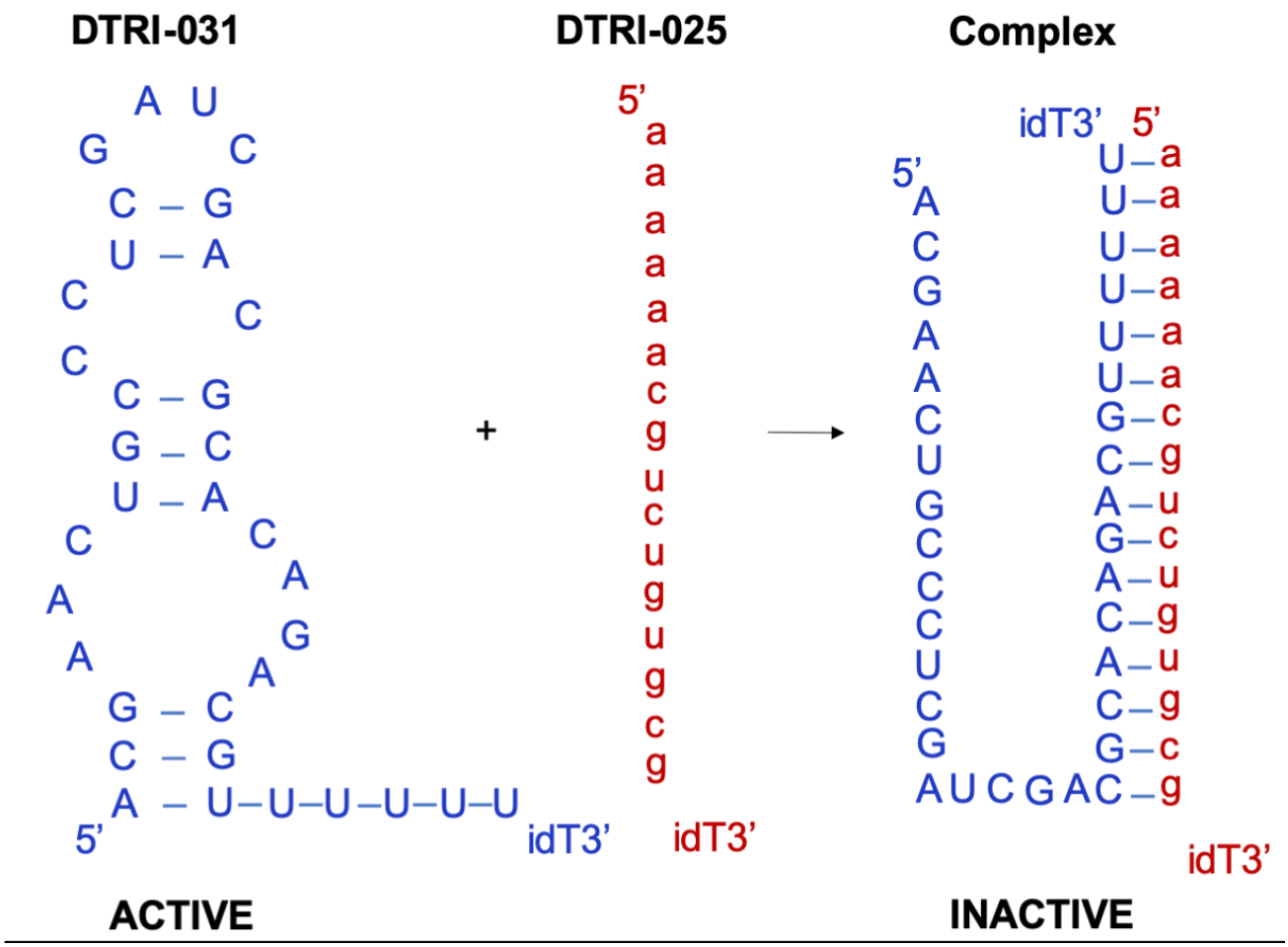


Figure 4: Inactivation of aptamer DRTI-031 by reverse oligonucleotide antidote DTRI-025 (Nimjee et al. 2020)

Table 1

Sex	Vehicle (5 min bolus, then 40 min infusion)
Male	0/3
Female	1/2
Total	1/5

Figure 5

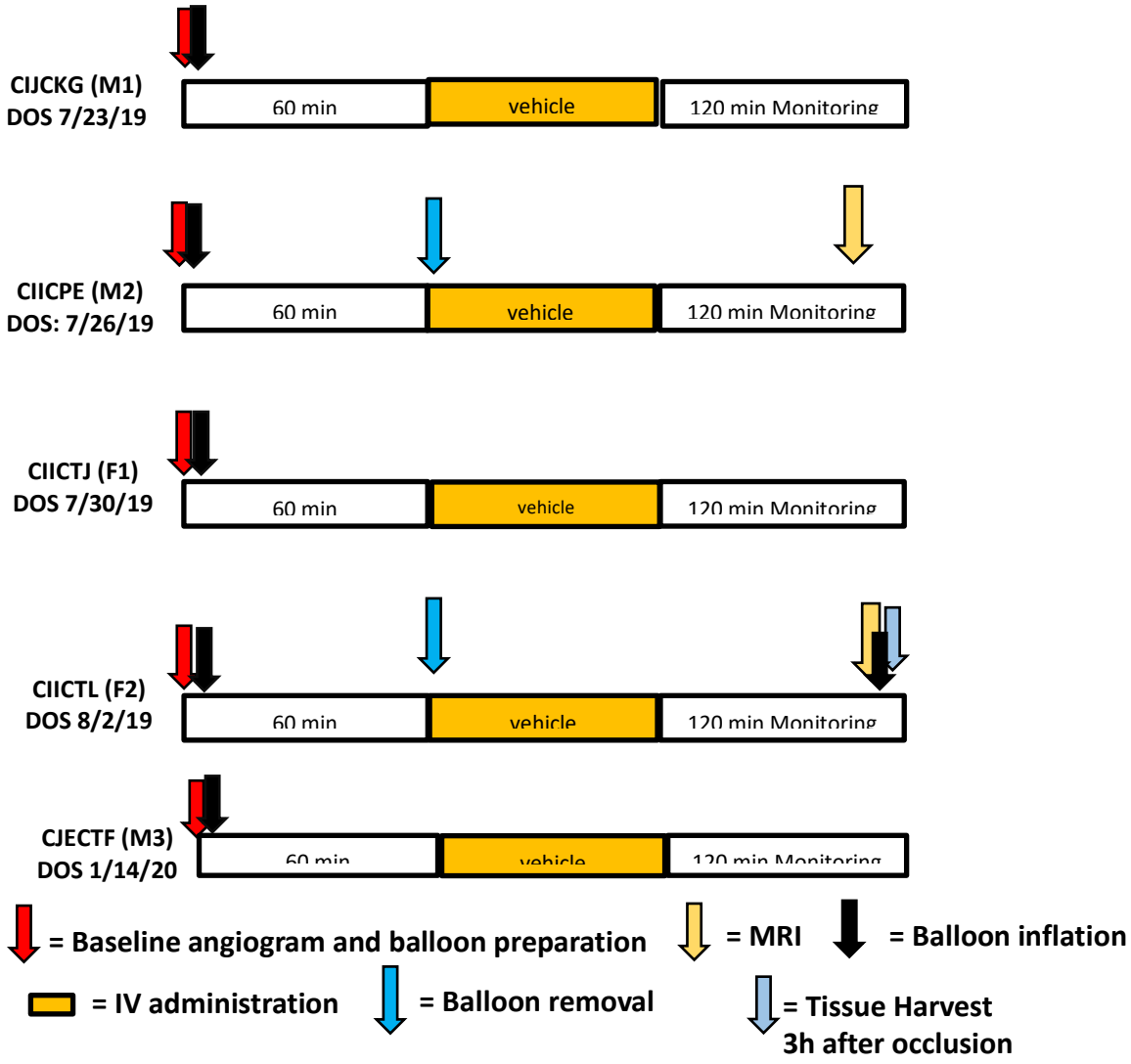

CIJCKG (M1)
DOS 7/23/19
60 min
vehicle
120 min Monitoring
CIICPE (M2)
DOS: 7/26/19
60 min
vehicle
120 min Monitoring
CIICTJ (F1)
DOS 7/30/19
60 min
vehicle
120 min Monitoring
CIICTL (F2)
DOS 8/2/19
60 min
vehicle
120 min Monitoring
CJECTF (M3)
DOS 1/14/20
60 min
vehicle
120 min Monitoring
= Baseline angiogram and balloon preparation
= MRI
= Balloon inflation
= IV administration
= Balloon removal
= Tissue Harvest
3h after occlusion

Figure 6

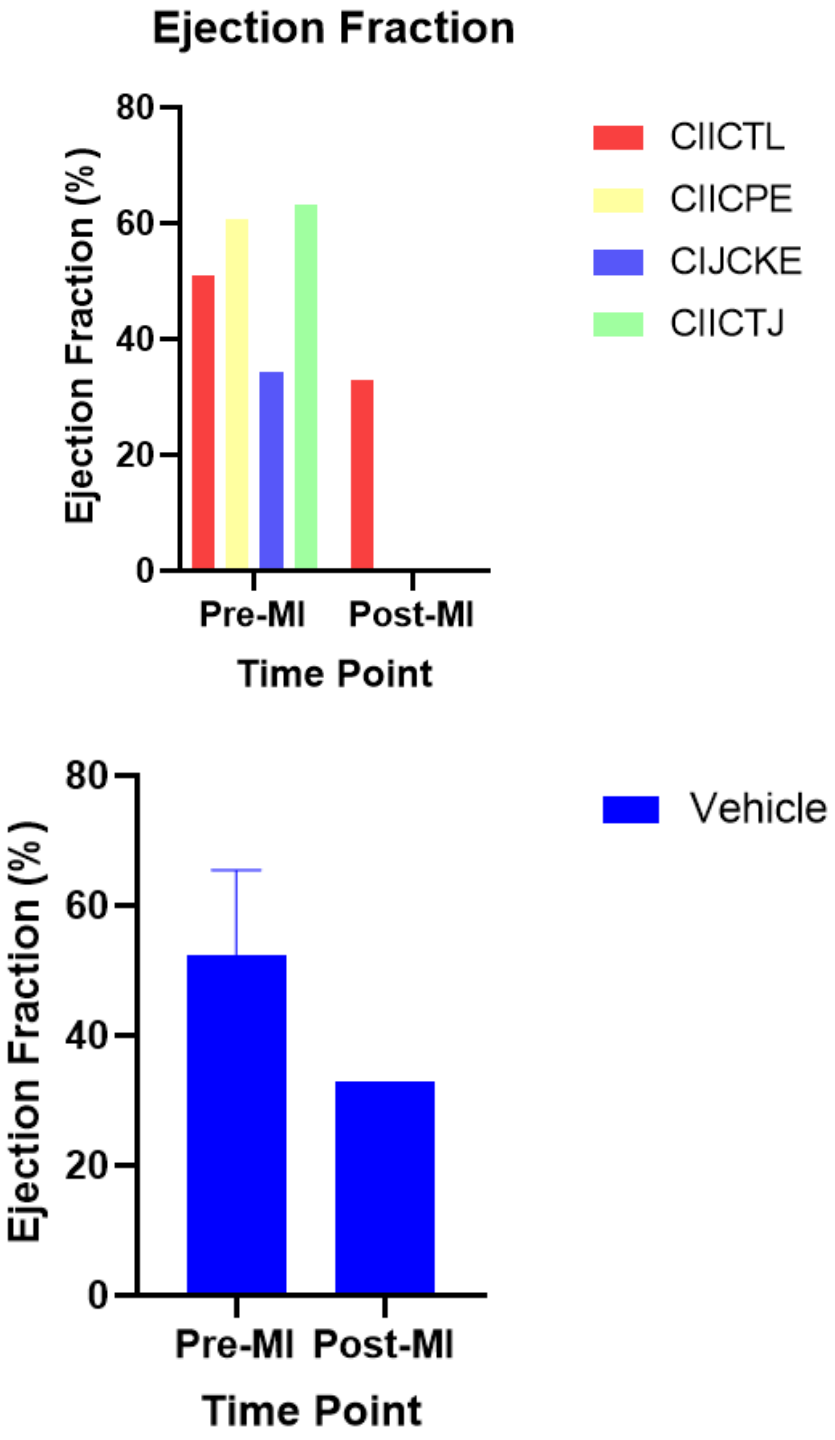

Ejection Fraction
Ejection Fraction (%)
80
60
40
20
0
Pre-MI
Post-MI
Time Point
CIICTL
CIICPE
CIJCKE
CIICTJ
Vehicle

Figure 7

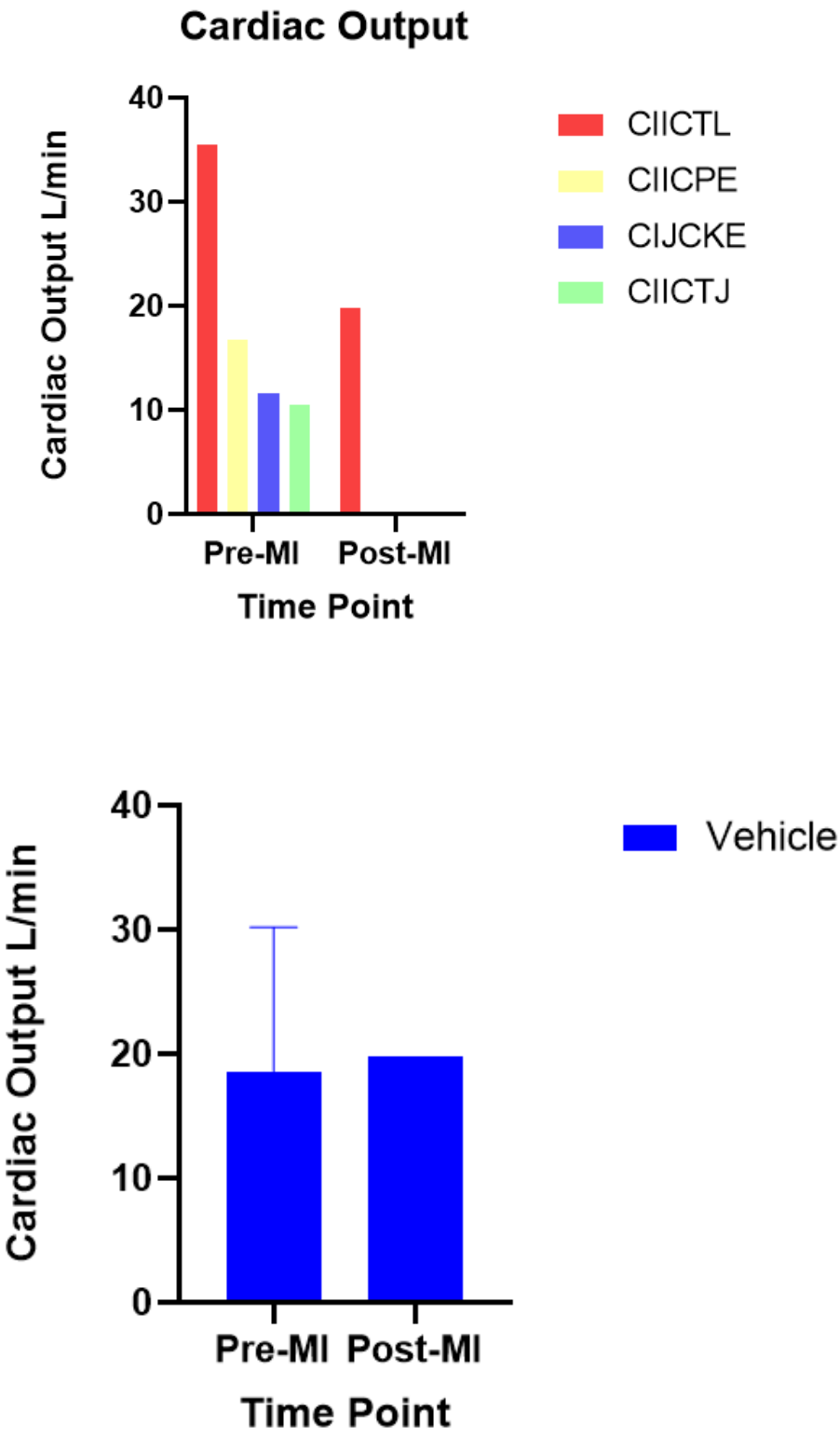

Cardiac Output
Cardiac Output L/min
40
30
20
10
0
Pre-MI
Post-MI
Time Point
CIICTL
CIICPE
CIJCKE
CIICTJ
Cardiac Output L/min
40
30
20
10
0
Pre-MI
Post-MI
Time Point
Vehicle

Figure 8

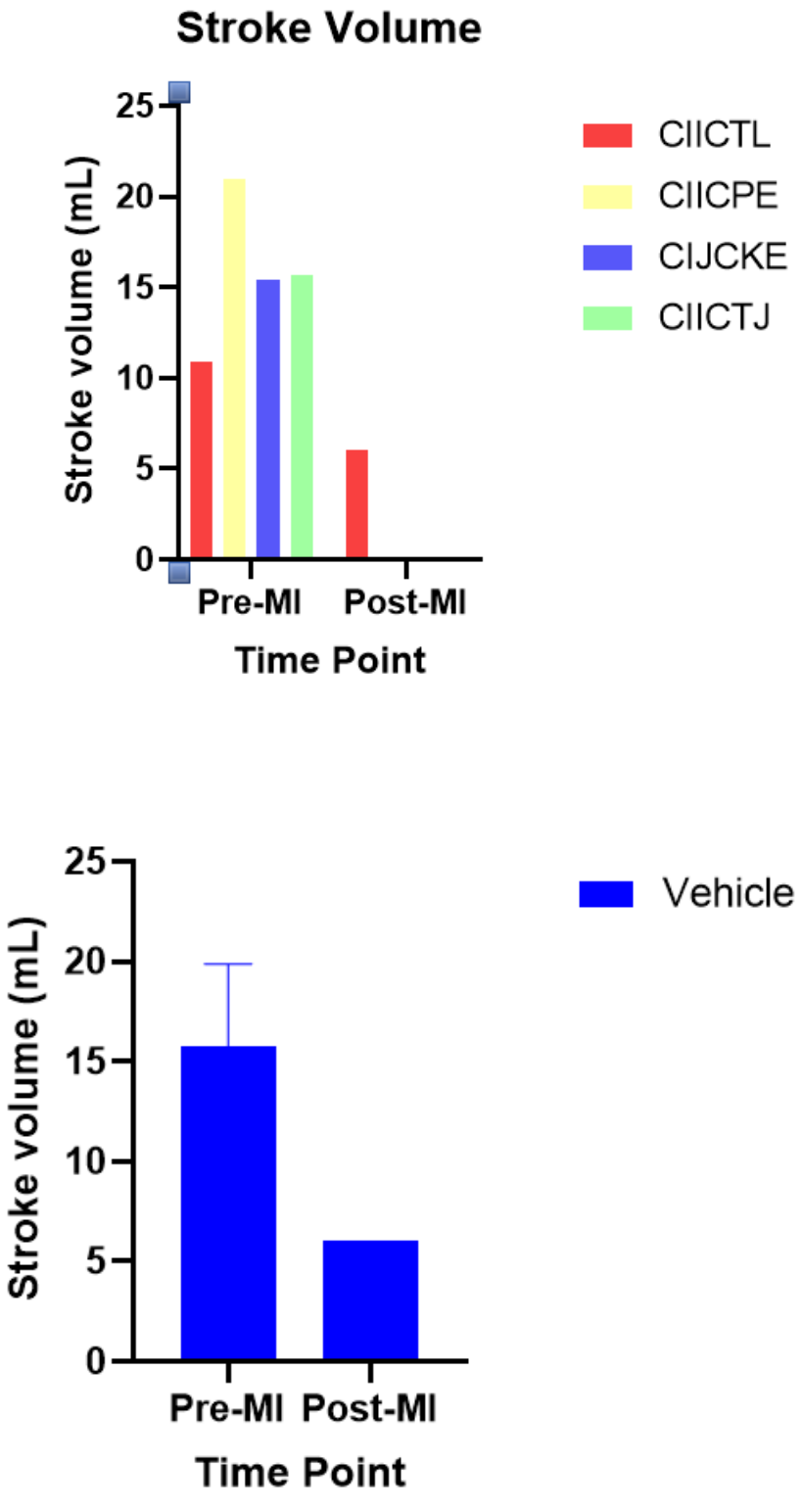

Stroke Volume
Stroke volume (mL)
25
20
15
10
5
0
Pre-MI
Post-MI
Time Point
CIICTL
CIICPE
CIJCKE
CIICTJ
Vehicle
Stroke volume (mL)
25
20
15
10
5
0
Pre-MI
Post-MI
Time Point

Figure 9

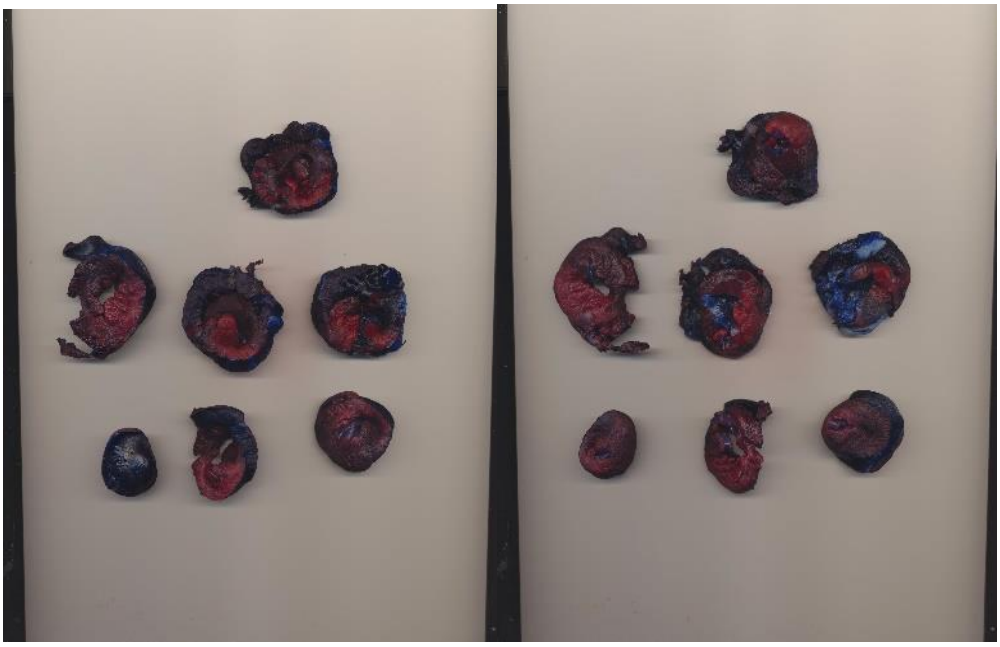


Figure 9: Dog #F2: CIICTL / Vehicle

Figure 10

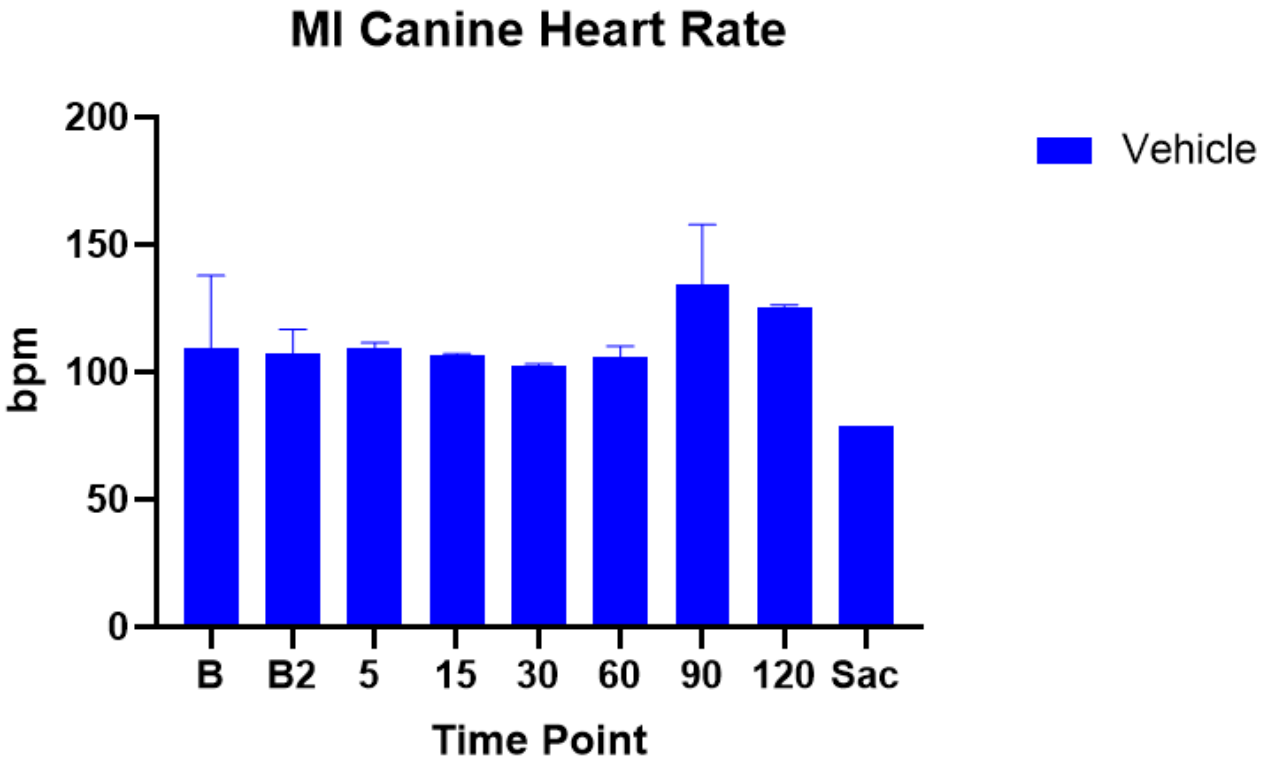

MI Canine Heart Rate
200
150
100
50
0
bpm
B
B2
5
15
30
60
90
120
Sac
Time Point
Vehicle

Figure 11

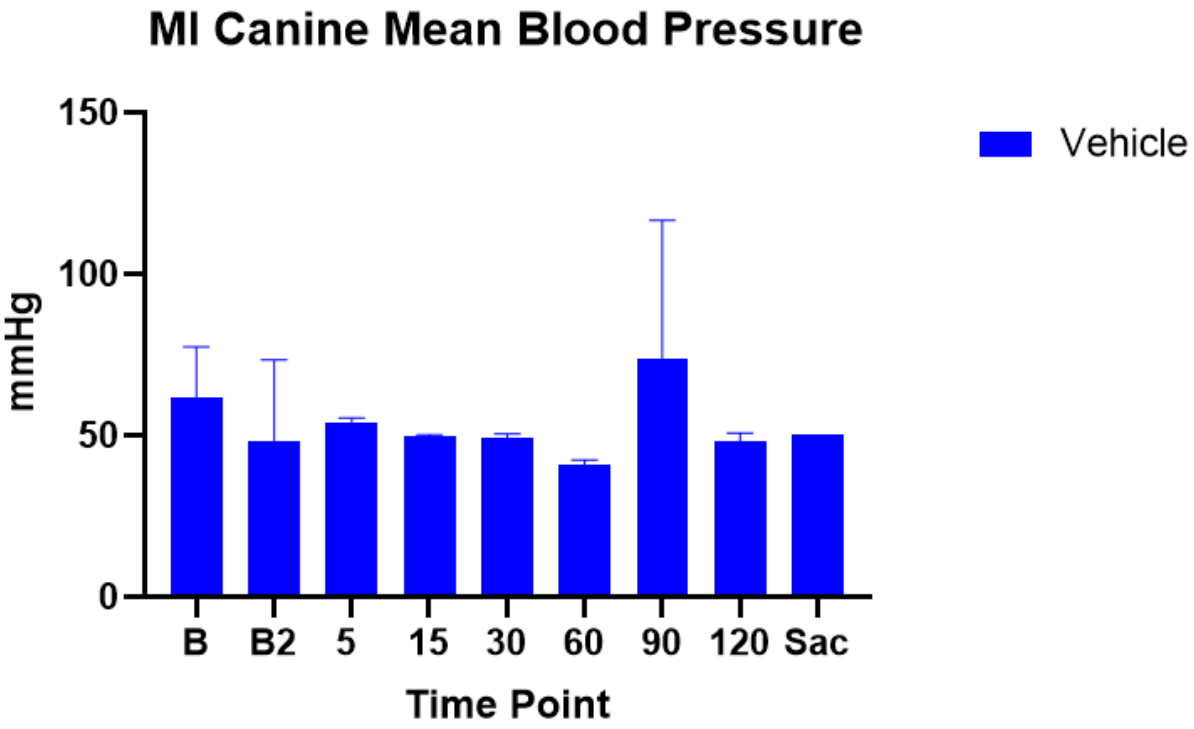

MI Canine Mean Blood Pressure
150
100
50
0
mmHg
B
B2
5
15
30
60
90
120
Sac
Time Point
Vehicle

Figure 12

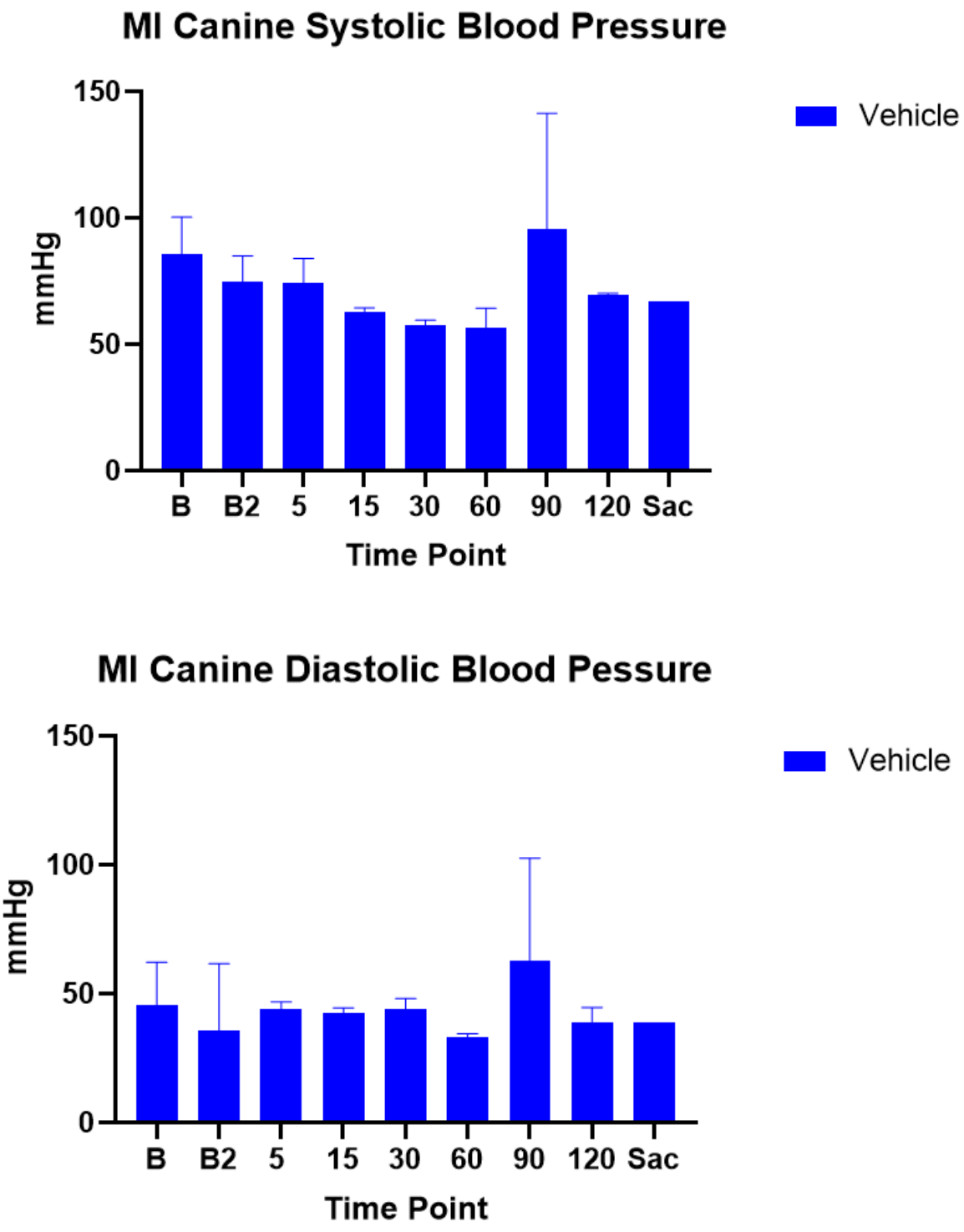

MI Canine Systolic Blood Pressure
150
100
50
0
mmHg
B
B2
5
15
30
60
90
120
Sac
Time Point
Vehicle
MI Canine Diastolic Blood Pessure
150
100
50
0
mmHg
B
B2
5
15
30
60
90
120
Sac
Time Point
Vehicle

Figure 13

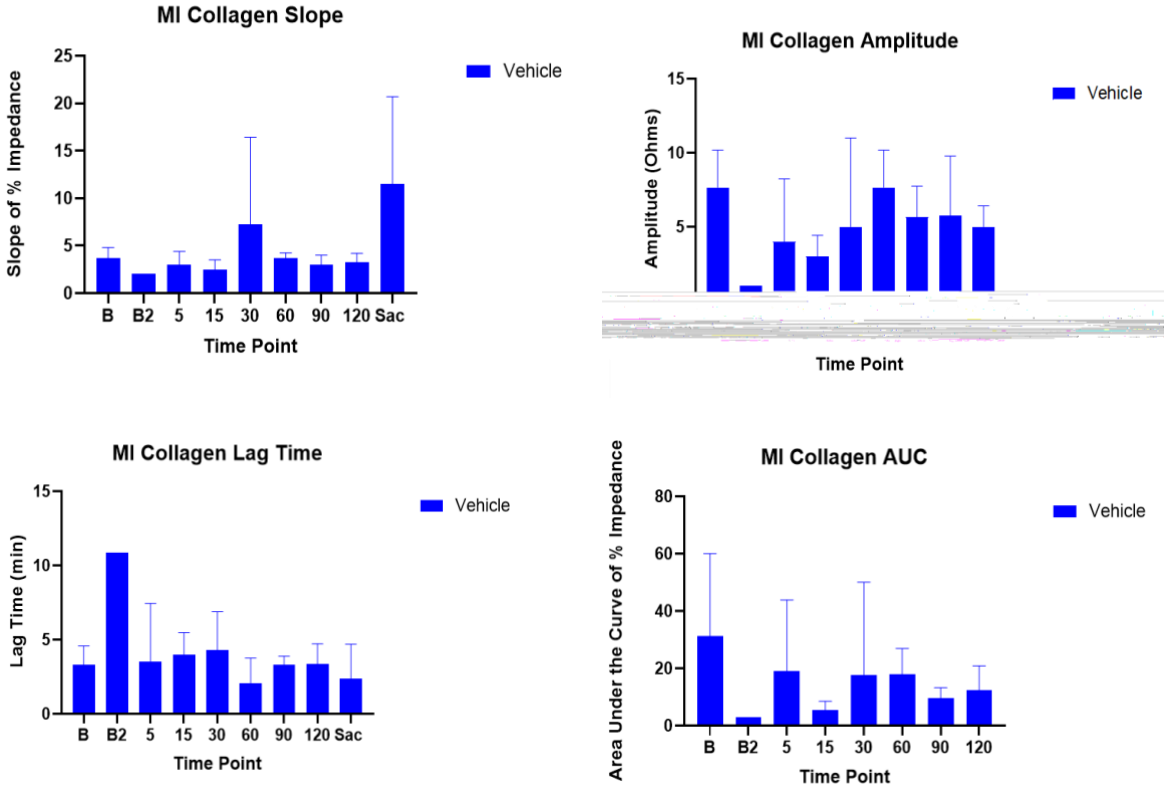

MI Collagen Slope
Slope of % Impedance
25
20
15
10
5
0
B B2 5 15 30 60 90 120 Sac
Time Point
Vehicle
MI Collagen Amplitude
Amplitude (Ohms)
15
10
5
Time Point
Vehicle
MI Collagen Lag Time
Lag Time (min)
15
10
5
0
B B2 5 15 30 60 90 120 Sac
Time Point
Vehicle
MI Collagen AUC
Area Under the Curve of % Impedance
80
60
40
20
0
B B2 5 15 30 60 90 120
Time Point
Vehicle

Figure 14

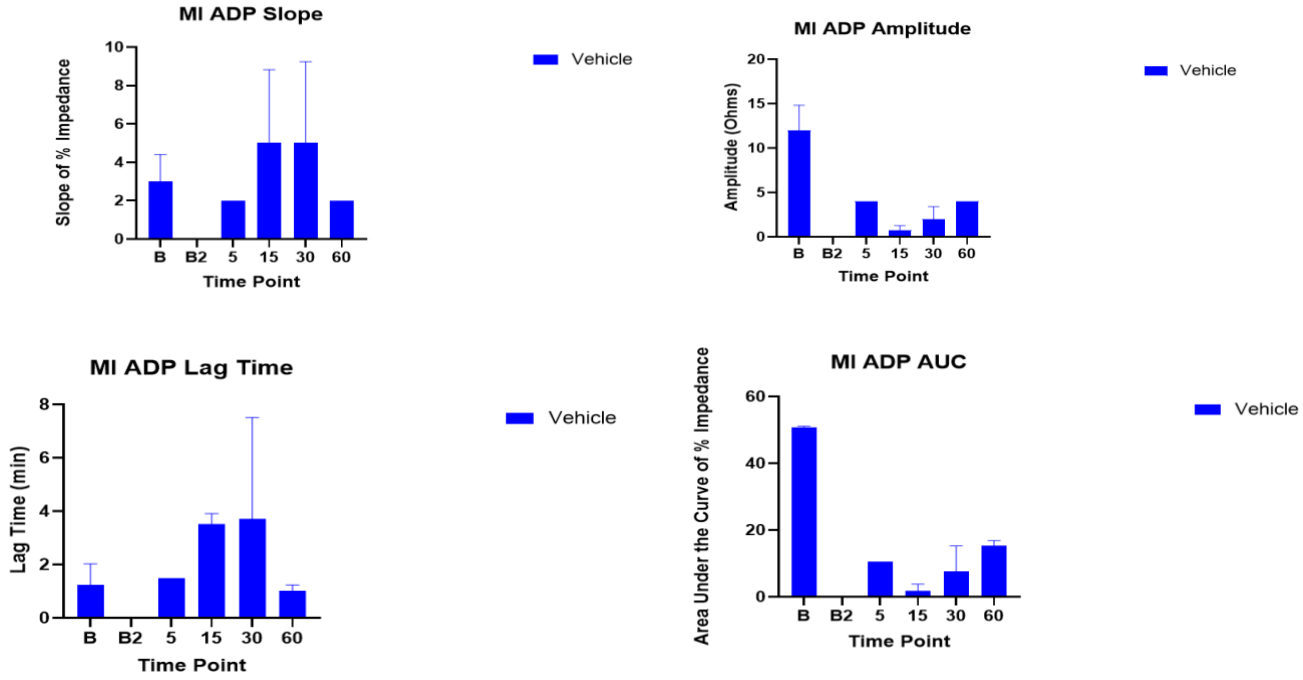
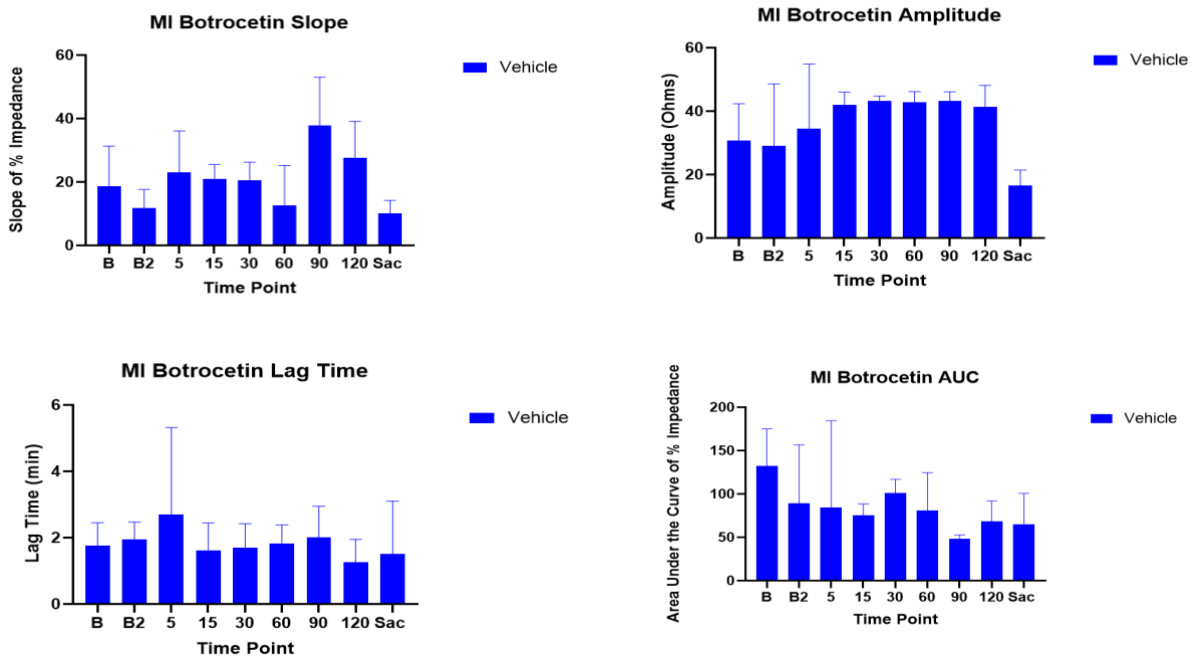

MI ADP Slope
Slope of % Impedance
10
8
6
4
2
0
B
B2
5
15
30
60
Time Point
Vehicle
MI ADP Amplitude
Amplitude (Ohms)
20
15
10
5
0
B
B2
5
15
30
60
Time Point
Vehicle
MI ADP Lag Time
Lag Time (min)
8
6
4
2
0
B
B2
5
15
30
60
Time Point
Vehicle
MI ADP AUC
Area Under the Curve of % Impedance
60
40
20
0
B
B2
5
15
30
60
Time Point
Vehicle

Figure 15


MI Botrocetin Slope
Slope of % Impedance
60
40
20
0
B B2 5 15 30 60 90 120 Sac
Time Point
Vehicle
MI Botrocetin Amplitude
Amplitude (Ohms)
60
40
20
0
B B2 5 15 30 60 90 120 Sac
Time Point
Vehicle
MI Botrocetin Lag Time
Lag Time (min)
6
4
2
0
B B2 5 15 30 60 90 120 Sac
Time Point
Vehicle
MI Botrocetin AUC
Area Under the Curve of % Impedance
200
150
100
50
0
B B2 5 15 30 60 90 120 Sac
Time Point
Vehicle

VII. Acknowledgements

First, I'd like to thank Dr. Shahid Nimjee, MD/PhD for his mentorship on this project. I want to thank Ms. Debra Wheeler, BS for her amazing support throughout my years in this lab and her guidance on this project. I also want to thank Dr. Surya Gynawali, PhD and Mr. Matthew Joseph for their efforts in specific data analysis and surgical assistance. Lastly, I'd like to thank all the previous and current members of the Nimjee Lab for their support. My time in this lab would not have been the same without any of these people. Their incredible drive and dedication for science pushed me to do more each day.

VIII. References

1. Heart Disease Facts. (2019, December 2). Retrieved March 11, 2020, from https://www.cdc.gov/heartdisease/facts.htm
2. Lu, L., Liu, M., Sun, R. *et al.* Myocardial Infarction: Symptoms and Treatments. *Cell Biochem Biophys* **72,** 865–867 (2015). https://doi.org/10.1007/s12013-015-0553-4
3. NHLBI. (n.d.). Heart Attack. Retrieved from https://www.nhlbi.nih.gov/health-topics/heart-attack
4. Sánchez-Hernández, C. D., Torres-Alarcón, L. A., González-Cortés, A., & Peón, A. N. (2020). Ischemia/Reperfusion Injury: Pathophysiology, Current Clinical Management, and Potential Preventive Approaches. *Mediators of Inflammation*, *2020*, 1–13. doi: 10.1155/2020/8405370
5. Guha, Santanu & Mookerjee, Soura & Lahiri, P & Mani, Sidhartha & Saha, Jayanta & Guha, Sharmila & Majumdar, Debdatta & Mandal, Md & Bhattacharya, Raja. (2011). A study of platelet aggregation in patients with acute myocardial infarction at presentation and after 48 hrs of initiating standard anti platelet therapy. Indian heart journal. 63. 409-13.
6. Bryckaert, M., Rosa, J. P., Denis, C. V., & Lenting, P. J. (2015). Of von Willebrand factor and platelets. *Cellular and molecular life sciences : CMLS*, *72*(2), 307–326. https://doi.org/10.1007/s00018-014-1743-8
7. Tomaiuolo, M., Brass, L. F., & Stalker, T. J. (2017). Regulation of Platelet Activation and Coagulation and Its Role in Vascular Injury and Arterial Thrombosis. *Interventional cardiology clinics*, *6*(1), 1–12. https://doi.org/10.1016/j.iccl.2016.08.001
8. Spiel, A. O., Gilbert, J. C., & Jilma, B. (2008). Von Willebrand Factor in Cardiovascular Disease. *Circulation*, *117*(11), 1449–1459. doi: 10.1161/circulationaha.107.722827

9. Rutten, B., Maseri, A., Cianflone, D., Laricchia, A., Cristell, N., Durante, A., … Roest, M. (2014). Plasma levels of active Von Willebrand factor are increased in patients with first ST-segment elevation myocardial infarction: A multicenter and multiethnic study. *European Heart Journal: Acute Cardiovascular Care*, *4*(1), 64–74. doi: 10.1177/2048872614534388
10. Nimjee SM. The potential of aptamers as anticoagulants. *Trends in Cardiovascular Medicine.* 01/2005;15(1):41-45. doi:10.1016/j.tcm.2005.01.002 19. 20. 21. 22.)
11. Flood, V. H., Slobodianuk, T. L., Keesler, D., Lohmeier, H. K., Fahs, S., Zhang, L., Simpson, P., & Montgomery, R. R. (2020). von Willebrand factor binding to myosin assists in coagulation. *Blood advances*, *4*(1), 174–180. https://doi.org/10.1182/bloodadvances.2019000533
12. Nimjee SM. Preclinical Development of a VWF Aptamer to Limit Thrombosis and Engender Arterial Recanalization of Occluded Vessels. *Molecular Therapy accepted* #MTJ-18-3228
13. Cai M. etal., Transgenic over expression of ectonucleotide triphosphate diphosphohydrolase-1 protects against murine myocardial ischemic injury. *Journal of Molecular and Cellular Cardiology.* 12/2011;51(6):927-935. doi: 10.1016/j.yjmcc.2011.09.003.
14. Kawecki C. Von willebrand factor and inflammation. *Journal of Thrombosis and Haemostasis.* 2017;15(7):1285-1294. doi: 10.1111/jth.13696.
15. Sonneveld MAH. Von willebrand factor and ADAMTS13 in arterial thrombosis: A systematic review and meta-analysis. *Blood Reviews.* 07/2014;28(4):167-178. doi: 10.1016/j.blre.2014.04.003.

16. Rakic M. Possible role of circulating endothelial cells in patients after acute myocardial infarction. *Medical Hypotheses.* 08/2018;117:42-46. doi: 10.1016/j.mehy.2018.06.005.
17. Regueiro A. Mobilization of endothelial progenitor cells in acute cardiovascular events in the PROCELL study: Time-course after acute myocardial infarction and stroke. *Journal of Molecular and Cellular Cardiology.* 03/2015;80:146-155. doi: 10.1016/j.yjmcc.2015.01.005.
18. Wang X. Kinetics of plasma von willebrand factor in acute myocardial infarction patients: A meta- analysis. *Oncotarget.* 10/2017;8(52):90371-90379. doi: 10.18632/oncotarget.20091
19. Andreotti, F., Roncaglioni, M., Hackett, D. R., Khan, M. I., Regan, T., Haider, A. W., … Maseri, A. (1990). Early coronary reperfusion blunts the procoagulant response of plasminogen activator inhibitor-1 and von Willebrand factor in acute myocardial infarction. *Journal of the American College of Cardiology*, *16*(7), 1553–1560. doi: 10.1016/0735-1097(90)90300-e
20. Ozawa K. Molecular imaging of VWF (von willebrand factor) and platelet adhesion in postischemic impaired microvascular reflow. *Circulation. Cardiovascular imaging.* 11/2018;11(11):e007913. doi: 10.1161/CIRCIMAGING.118.007913.
21. Wheeler DG. Transgenic swine: Expression of human CD39 protects against myocardial injury. *Journal of Molecular and Cellular Cardiology.* 05/2012;52(5):958-961.
22. Talukder MAH. Reduced SERCA2a converts sub-lethal myocardial injury to infarction and affects postischemic functional recovery. *Journal of molecular and cellular cardiology.* 02/2009;46(2):285-287. doi: 10.1016/j.yjmcc.2008.10.026.).

23. Smith SB. Impact of cardiac-specific expression of CD39 on myocardial infarct size in mice. *Life sciences* (1973). 06/2017;179:54-59. doi: 10.1016/j.lfs.2016.10.016.
24. Nimjee SM. Rapidly regulating platelet activity in vivo with an antidote controlled platelet inhibitor. *Molecular therapy*. 02/2012;20(2):391-397. doi: 10.1038/mt.2011.226.
25. American Heart Association. (n.d.). Ejection Fraction Heart Failure Measurement. Retrieved from https://www.heart.org/en/health-topics/heart-failure/diagnosing-heart-failure/ejection-fraction-heart-failure-measurement
26. Vincent J. L. (2008). Understanding cardiac output. *Critical care (London, England)*, *12*(4), 174. https://doi.org/10.1186/cc6975
27. Manning, W. J., & Pennell, D. J. (2019). *Cardiovascular magnetic resonance: a companion to Braunwalds heart disease*. Philadelphia, PA: Elsevier.
28. Chrono-log Corporation. (n.d.). Retrieved from http://www.chronolog.com/
29. Ruggeri, Z. M. The role of von Willebrand factor in thrombus formation. *Thromb. Res.* 120, S5–S9 (2007).
30. Huttinger AL. Ferric chloride-induced canine carotid artery thrombosis: A large animal model of vascular injury. *Journal of visualized experiments*. 2018(139). doi: 10.3791/57981
31. Paul A. McEwan, Robert K. Andrews, Jonas Emsley; Glycoprotein Ibα inhibitor complex structure reveals a combined steric and allosteric mechanism of von Willebrand factor antagonism. *Blood* 2009; 114 (23): 4883–4885. doi: https://doi.org/10.1182/blood-2009-05-224170

32. Li, R., & Emsley, J. (2013). The organizing principle of the platelet glycoprotein Ib-IX-V complex. *Journal of Thrombosis and Haemostasis*, *11*(4), 605–614. doi: 10.1111/jth.12144

33. Vickers, J. D. (1999). Binding of polymerizing fibrin to integrin alpha IIb beta3 on chymotrypsin-treated rabbit platelets decreases phosphatidylinositol 4,5-bisphosphate and increases cytoskeletal actin. *Platelets*, *10*(4), 228–237. doi: 10.1080/09537109976077

34. Calvete, J. J. (1995). On the Structure and Function of Platelet Integrin αIIbβ3, the Fibrinogen Receptor. Proceedings of the Society for Experimental Biology and Medicine, 208(4), 346–360. https://doi.org/10.3181/00379727-208-43863A